

WHAT IS CLAIMED IS:

Claim 1. A method for the imaging of a particular volume of plant or animal tissue, wherein the plant or animal tissue contains at least one photo-active molecular agent. the method comprising the steps of:

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- (a) treating the particular volume of the plant or animal tissue with light sufficient to promote a simultaneous two-photon excitation of the photo-active molecular agent contained in the particular volume of the plant or animal tissue;
 - (b) photo-activating at least one of the at least one photo-active molecular agent in the particular volume of the plant or animal tissue, thereby producing at least one photo-activated molecular agent, wherein the at least one photo-activated molecular agent emits energy;
 - (c) detecting the energy emitted by the at least one photo-activated molecular agent; and
 - (d) producing a detected energy signal which is characteristic of the particular volume of plant or animal tissue.

Claim 2. The method of Claim 1 wherein the light sufficient to promote a simultaneous two-photon excitation of the at least one photo-active molecular agent is laser light.

Claim 3. The method of Claim 1 wherein the light sufficient to promote a simultaneous two-photon excitation of the photo-active molecular agent is a focused beam of light.

Claim 4. The method of Claim 4 wherein the focused beam of light is focused laser light.

Claim 5. The method of Claim 1 further including a first step of treating the plant or animal tissue with at least one photo-active molecular agent, wherein the particular volume of the plant or animal tissue retains at least a portion of the at least one photo-active molecular agent.

Claim 6. The method of Claim 5 wherein the at least one photo-active molecular agent is selected from the group consisting of psoralen, 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), 4,5',8-trimethylpsoralen (TMP), 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT), 5-chloromethyl-8-methoxypsoralen (HMT), angelicin (isopsoralen), 5-methylangelicin (5-MIP), 3-carboxypsoralen, porphyrin, haematoporphyrin derivative (HPD), photofrin II, benzoporphyrin derivative (BPD), protoporphyrin IX (PpIX), dye haematoporphyrin ether (DHE), polyhaematoporphyrin esters (PHE), 13,17-N,N,N-dimethylethylethanolamine ester of protoporphyrin (PH1008), tetra(3-hydroxyphenyl)-porphyrin (3-THPP), tetraphenylporphyrin monosulfonate (TPPS1), tetraphenylporphyrin disulfonate (TPPS2a), dihaematoporphyrin ether, mesotetraphenylporphyrin, mesotetra(4N-methylpyridyl)porphyrin (T4MpyP), octa-(4-*tert*-butylphenyl)tetrapyrazinoporphyrazine (OTPP), phthalocyanine, tetra-(4-*tert*-butyl)phthalocyanine (t_4 -PcH₂), tetra-(4-*tert*-butyl)phthalocyanatomagnesium (t_4 -PcMg), chloroaluminum sulfonated phthalocyanine (CASPc), chloroaluminum phthalocyanine tetrasulfate (AlPcTS), mono-sulfonated aluminum phthalocyanine (AlSPc), di-sulfonated aluminum phthalocyanine (AlS2Pc), tri-sulfonated aluminum phthalocyanine (AlS3Pc), tetra-sulfonated aluminum phthalocyanine

(AlS4Pc), silicon phthalocyanine (SiPc IV), zinc II phthalocyanine (ZnPc), bis(di-isobutyl octadecylsiloxy)silicon 2,3-naphthalocyanine (isoBOSINC), germanium IV octabutoxyphthalocyanine (GePc), rhodamine 101 (Rh-101), rhodamine 110 (Rh-110), rhodamine 123 (Rh-123), rhodamine 19 (Rh-19), rhodamine 560 (Rh-560), rhodamine 575 (Rh-575),
20 rhodamine 590 (Rh-590), rhodamine 610 (Rh-610), rhodamine 640 (Rh-640), rhodamine 6G (Rh-6G), rhodamine 700 (Rh-700), rhodamine 800 (Rh-800), rhodamine B (Rh-B), sulforhodamine 101, sulforhodamine 640, sulforhodamine B, coumarin 1, coumarin 2, coumarin 4, coumarin 6, coumarin 6H, coumarin 7, coumarin 30, coumarin 47, coumarin 102, coumarin 106, coumarin 120, coumarin 151, coumarin 152, coumarin 152A, coumarin 153, coumarin 311, coumarin 307,
25 coumarin 314, coumarin 334, coumarin 337, coumarin 343, coumarin 440, coumarin 450, coumarin 456, coumarin 460, coumarin 461, coumarin 466, coumarin 478, coumarin 480, coumarin 481, coumarin 485, coumarin 490, coumarin 500, coumarin 503, coumarin 504, coumarin 510, coumarin 515, coumarin 519, coumarin 521, coumarin 522, coumarin 523, coumarin 535, coumarin 540, coumarin 540A, coumarin 548, 5-ethylamino-9-
30 diethylaminobenzo[a]phenoxazinium (EtNBA), 5-ethyl-amino-9-diethyl-aminobenzo[a]phenothiazinium (EtNBS), 5-ethylamino-9-diethylaminobenzo[a]pheno-selenazinium (EtNBSe), chlorpromazine, chlorpromazine derivatives, chlorophyll derivatives, bacteriochlorophyll derivatives, metal-ligand complexes, tris(2,2'-bipyridine)ruthenium (II) dichloride (RuBPY), tris(2,2'-bipyridine)rhodium (II) dichloride (RhBPY), tris(2,2'-
35 bipyridine)platinum (II) dichloride (PtBPY), pheophorbide a, merocyanine 540, vitamin D, 5-amino-laevulinic acid, photosan, chlorin e6, chlorin e6 ethylenediamide, mono-L-aspartyl chlorin e6, phenoxazine Nile blue derivatives, stilbene, stilbene derivatives, 4-(N-(2-hydroxyethyl)-N-

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methyl)-aminophenyl)-4'-(6-hydroxyhexylsulfonyl)stilbene (APSS), and standard biological dyes and stains.

Claim 7. The method of Claim 5 wherein the at least one photo-active molecular agent is at least one biogenic photo-active molecular agent that is specific to a particular tissue within the particular volume of plant or animal tissue.

Claim 8. The method of Claim 7 wherein the at least one biogenic photo-active molecular agent includes a segment selected from the group consisting of DNA, RNA, amino acids, proteins, antibodies, ligands, haptens, carbohydrate receptors or complexing agents, lipid receptors or complexing agents, protein receptors or complexing agents, chelators, and encapsulating vehicles.

Claim 9. The method of Claim 8 wherein the at least one biogenic photo-active molecular agent further includes a segment which is photo-activated when subject to light sufficient to promote a simultaneous two-photon excitation.

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- and further including the steps of:

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Claim 11.

Claim 12. The method of Claim 10 wherein the demodulated energy signal which is characteristic of the particular volume of the plant or animal tissue represents a change in lifetime of at least one photo-activated molecular agent present in the particular volume of the plant or animal tissue.

Claim 13. A method for the imaging of a particular volume of material, wherein the material contains at least one photo-active molecular agent, the method comprising the steps of:

- (a) treating the particular volume of the material with light sufficient to promote a simultaneous two-photon excitation of at least one of the at least one photo-active molecular agent contained in the particular volume of the material;
- (b) photo-activating the at least one photo-active molecular agent in the particular volume of the material, thereby producing at least one photo-activated molecular agent, wherein the at least one photo-activated molecular agent emits energy;
- (c) detecting the energy emitted by the at least one photo-activated molecular agent; and
- (d) producing a detected energy signal which is characteristic of the particular volume of the material.

Claim 14. The method of Claim 13 wherein the material is selected from the group consisting of plant tissue and animal tissue.

Claim 15. The method of Claim 13 wherein the light sufficient to promote a simultaneous two-photon excitation of the at least one photo-active molecular agent is laser light.

Claim 16. The method of Claim 13 wherein the light sufficient to promote a simultaneous two-photon excitation of the at least one photo-active molecular agent is a focused beam of light.

Claim 17. The method of Claim 16 wherein the focused beam of light is focused laser light.

Claim 18. The method of Claim 13 further including a first step of treating the material with at least one photo-active molecular agent, wherein the particular volume of the material retains at least a portion of the at least one photo-active molecular agent.

Claim 19. The method of Claim 18 wherein the at least one photo-active molecular agent is selected from the group consisting of psoralen, 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), 4,5',8-trimethylpsoralen (TMP), 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT), 5-chloromethyl-8-methoxypsoralen (HMT), angelicin (isopsoralen), 5-methylangelicin (5-MIP), 3-carboxypsoralen, porphyrin, haematoporphyrin derivative (HPD), photofrin II, benzoporphyrin derivative (BPD), protoporphyrin IX (PpIX), dye haematoporphyrin ether (DHE), polyhaematoporphyrin esters (PHE), 13,17-N,N,N-dimethylethylethanolamine ester of protoporphyrin (PH1008), tetra(3-hydroxyphenyl)-porphyrin (3-THPP), tetraphenylporphyrin monosulfonate (TPPS1), tetraphenylporphyrin disulfonate (TPPS2a), dihaematoporphyrin ether, mesotetraphenylporphyrin, mesotetra(4N-methylpyridyl)porphyrin (T4MpyP), octa-(4-*tert*-

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(t_4 -PcH₂), tetra-(4-*tert*-butyl)phthalocyanatomagnesium (t_4 -PcMg), chloroaluminum sulfonated phthalocyanine (CASPC), chloroaluminum phthalocyanine tetrasulfate (AlPcTS), mono-sulfonated aluminum phthalocyanine (AlSPc), di-sulfonated aluminum phthalocyanine (AlS2Pc), tri-
15 sulfonated aluminum phthalocyanine (AlS3Pc), tetra-sulfonated aluminum phthalocyanine (AlS4Pc), silicon phthalocyanine (SiPc IV), zinc II phthalocyanine (ZnPc), bis(di-isobutyl octadecylsiloxy)silicon 2,3-naphthalocyanine (isoBOSINC), germanium IV octabutoxyphthalocyanine (GePc), rhodamine 101 (Rh-101), rhodamine 110 (Rh-110), rhodamine 123 (Rh-123), rhodamine 19 (Rh-19), rhodamine 560 (Rh-560), rhodamine 575 (Rh-575), rhodamine 590 (Rh-590), rhodamine 610 (Rh-610), rhodamine 640 (Rh-640), rhodamine 6G (Rh-6G), rhodamine 700 (Rh-700), rhodamine 800 (Rh-800), rhodamine B (Rh-B), sulforhodamine 101, sulforhodamine 640, sulforhodamine B, coumarin 1, coumarin 2, coumarin 4, coumarin 6, coumarin 6H, coumarin 7, coumarin 30, coumarin 47, coumarin 102, coumarin 106, coumarin 120, coumarin 151, coumarin 152, coumarin 152A, coumarin 153, coumarin 311, coumarin 307, coumarin 314, coumarin 334, coumarin 337, coumarin 343, coumarin 440, coumarin 450, coumarin 456, coumarin 460, coumarin 461, coumarin 466, coumarin 478, coumarin 480, coumarin 481, coumarin 485, coumarin 490, coumarin 500, coumarin 503, coumarin 504, coumarin 510, coumarin 515, coumarin 519, coumarin 521, coumarin 522, coumarin 523, coumarin 535, coumarin 540, coumarin 540A, coumarin 548, 5-ethylamino-9-
30 diethylaminobenzo[a]phenoxazinium (EtNBA), 5-ethyl-amino-9-diethylaminobenzo[a]phenothiazinium (EtNBS), 5-ethylamino-9-diethylaminobenzo[a]phenoselenazinium (EtNBSe), chlorpromazine, chlorpromazine derivatives, chlorophyll derivatives, bacteriochlorophyll derivatives, metal-ligand complexes, tris(2,2'-bipyridine)ruthenium (II)

35 bacteriochlorophyll derivatives, metal-ligand complexes, tris(2,2'-bipyridine)ruthenium (II) dichloride (RuBPY), tris(2,2'-bipyridine)rhodium (II) dichloride (RhBPY), tris(2,2'-bipyridine)platinum (II) dichloride (PtBPY), pheophorbide a, merocyanine 540, vitamin D, 5-amino-laevulinic acid, photosan, chlorin e6, chlorin e6 ethylenediamide, mono-L-aspartyl chlorin e6, phenoxazine Nile blue derivatives, stilbene, stilbene derivatives, 4-(N-(2-hydroxyethyl)-N-methyl)-aminophenyl)-4'-(6-hydroxyhexylsulfonyl)stilbene (APSS), and standard biological dyes and stains.

Claim 20. The method of Claim 18 wherein the at least one photo-active molecular agent is at least one biogenic photo-active molecular agent that is specific to a particular substance within the particular volume of material.

Claim 21. The method of Claim 20 wherein the at least one biogenic photo-active molecular agent includes a segment selected from the group consisting of DNA, RNA, amino acids, proteins, antibodies, ligands, haptens, carbohydrate receptors or complexing agents, lipid receptors or complexing agents, protein receptors or complexing agents, chelators, and encapsulating vehicles.

Claim 22. The method of Claim 21 wherein the at least one biogenic photo-active molecular agent further includes a segment which is photo-activated when subject to light sufficient to promote a simultaneous two-photon excitation.

Claim 23. The method of Claim 13 wherein the step of treating the particular volume of the material with light sufficient to promote a simultaneous two-photon excitation of the at least one photo-active molecular agent contained in the particular volume of the material includes the steps of:

(a1) modulating light from a light source with a particular type of modulation, thereby producing a modulated light; and

(a2) treating the particular volume of the material with the modulated light sufficient to promote a simultaneous two-photon excitation of the at least one photo-active molecular agent contained in the particular volume of the material;

and further including the steps of:

(e) demodulating the detected energy signal with the particular type of modulation; and

(f) producing a demodulated energy signal which is characteristic of the particular volume of the material.

Claim 24. The method of Claim 23 wherein the step of demodulating the detected energy signal with the particular type of modulation includes demodulating the detected energy signal at a frequency twice that of the particular type of modulation, thereby detecting the second harmonic of the particular type of modulation.

Claim 25. The method of Claim 23 wherein the demodulated energy signal which is characteristic of the particular volume of the material represents a change in lifetime of at least one photo-activated molecular agent present in the particular volume of the material.